

# ***Flu-M<sup>3</sup>***

*(Influenza Mucosal Molecular Mechanisms)*

## ***Nasal and systemic immune responses to nasal influenza vaccine***

**Kinetics of Mucosal and Systemic Immune Responses to Intranasal Live Attenuated Influenza Vaccine (LAIV)**

**V1.0**

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<b>Name &amp; Role</b>	<b>Date</b>	<b>Signature</b>

## **Study Management Group**

Chief Investigator (CI): Professor Peter Openshaw

Co-investigators: Prof Trevor Hansel, Prof Robin Shattock, Dr Ryan Thwaites

Statisticians: Mrs Tanushree Tunstall, Dr Ryan Thwaites

## **Study Coordination Centre**

For general queries, supply of trial documentation, and collection of data, please contact:

Study Coordinator: Professor Peter Openshaw

Address: Room 353, Department of Respiratory Medicine, National Heart and Lung Institute, Medical School Building, St Mary's Hospital, Paddington, London, W2 1PG

Email: [p.openshaw@imperial.ac.uk](mailto:p.openshaw@imperial.ac.uk)

Tel: 020 7594 3854

Fax: 02033125751

## **Clinical Queries**

Clinical queries should be direct to Peter Openshaw, who will direct the query to the appropriate member of the study team.

## **Sponsor**

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:

Joint Research Compliance Office

Imperial College London and Imperial College Healthcare NHS Trust

Room 221, Level 2, Medical School Building

Norfolk Place

London, W2 1PG

Tel: 020 7594 1862

<http://www3.imperial.ac.uk/clinicalresearchgovernanceoffice>

## Funder

The study is funded by the National Institute for Health Research Imperial Biomedical Research Cluster (NIHR-BRC).

This protocol describes the *Flu-M<sup>3</sup>* study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. Any such amendments will be circulated to investigators in the study.

Problems relating to this trial should be referred, in the first instance, to the study coordination centre.

This trial will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

## Key Information

**Participant population:** Young Adults (18-30 years; n=85 with n=40 receiving vaccination)

**Scientific Aims:** Characterise mucosal and systemic influenza antibodies (IgG and IgA) induced by vaccination, viral shedding, innate and cellular immune responses

**Principal Investigator:** Professor Peter Openshaw

**Sponsor:** Imperial College London

**Site:** Imperial Clinical Respiratory Research Unit, 1<sup>st</sup> Floor, Mint Wing, St Mary's Hospital, Imperial College London

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## Abstract

Intranasal live attenuated influenza vaccine (LAIV; trade name FluMist/Fluenz-Tetra, manufactured by AstraZeneca/Medimmune) is the standard influenza vaccine given to children aged 2-17 years of age in the UK. It is also licensed to be given to adults up to the age of 49 years in the USA. The systems biology of the human blood response to influenza vaccines has been studied in great detail, but there is a paramount need to study innate and specific, soluble and cellular immune responses at the nasal mucosal site of influenza infection. In this way we hope to determine correlates of efficacy and vaccine take in serum and nasal mucosal lining fluid (MLF).

We wish to take serial samples prior to vaccination and at intervals up to day 28 post-vaccination to establish the kinetics of the nasal mucosal and blood systemic response to LAIV in young adults aged 18-30 years (n=40). In the nose we shall measure viral load, soluble mediators of inflammation and antibodies (humoral immunity) in mucosal lining fluid; while cellular immune responses and serology will be assessed in blood samples. Imperial College London (ICL) has been involved in the development of novel methods of non-invasive precision mucosal sampling, including absorption of MLF from the nose by nasosorption. ICL has also developed assays for influenza-specific IgA by ELISA, and we hope to compare against a repertoire of assays for serology in patients after LAIV administration.

The study involves precisely assessing mucosal and systemic immune responses to the LAIV nasal vaccine. The primary endpoint will be based on nasal mucosal levels of IgA and IgG antibodies to the 4 constituent viral subtypes in LAIV: measured by ELISA and multiplex immunoassay (Mesoscale Diagnostics) and expressed as seroconversion rates, geometric mean titre (GMT) changes, and geometric mean fold rises (GMFR). The secondary endpoints will be: (1) haemagglutination inhibition (HAI) titres measured in serum and the nose, (2) influenza pseudotype neutralisation by antibodies in serum and the nose, (3) nasal cytokine and chemokine levels and (4) nasal viral load.

It is thought that the immune response to LAIV in an individual is mediated by a combination of mucosal and systemic factors, involving innate and specific mechanisms that have different kinetics, and various cell types. By understanding the molecular and cellular basis of the nasal mucosal response to LAIV, we hope to be able to identify key molecular signatures and biomarkers associated with LAIV responses, and to assess protective pathways that could be stimulated by novel vaccines. The nasal vaccine challenge model could be used to test other new vaccines, and proceed to rational development of improved vaccines for influenza and other diseases. Furthermore nasal mucosal methods could be used in the clinic to identify subjects who have responded poorly to vaccines, or to assess vaccine efficacy in large populations.

## **Introduction**

### **Influenza**

Influenza viruses cause a continuous threat to global health, mutating and spreading in both human and animal populations. In the period from March to May 2009 a novel swine-origin influenza A (H1N1) virus emerged in Mexico and the United States (1, 2). Most cases were acute and self-limiting among children and young adults (2), although the global mortality over the first 12 months has been estimated at over 200,000 respiratory deaths (3). Those with severe illness typically presented with viral pneumonitis or pneumonia, sometimes complicated by multi-organ failure.

By measuring cytokines and chemokines in the serum of patients with pH1N1 influenza, it has been found that there can be over exuberant immune reactions to virus infection in severe flu, with very high levels of cytokines such as interferons and even a “cytokine storm” (4-8). Some studies have highlighted the importance of particular biomarkers: such as HMGB1 in H1N1 influenza-associated encephalopathy (9), and IL-6 in severe pH1N1 influenza (10). In studies of fatal cases of H1N1 the presence of viral pneumonia and immunolocalisation of viral antigen with diffuse alveolar damage has been noted (11, 12).

### **Influenza and Asthma**

The Influenza Clinical Information Network (FLU-CIN) recently reported the differences between asthmatics and non-asthmatics in 1520 patients hospitalised with influenza A infection across 75 UK hospitals (13). This study noted that patients with asthma (present in 25.3%) were less likely to have a severe outcome, despite more asthmatics requiring oxygen on admission. These investigators suggested that the association of asthma with less severe outcomes was due to corticosteroid use and earlier hospital admission. The better outcome of asthma patients when hospitalised with influenza has also been observed elsewhere (14, 15). Despite this, a global pooled analysis of H1N1 found that asthma was the major comorbidity associated with severe outcomes (16), as noted in a study of hospitalized US patients from April-June 2009 (17). These studies reflect the complex association between asthma and influenza severity.

### **IFN and Type 2 inflammation in Asthma**

Abnormal airway mucosal inflammatory responses are a feature of asthma (18), and an expanding number of asthma phenotypes are now recognised at a clinical and molecular level (19). Interferon (IFN) responses in asthmatics are controversial, since description of IFN deficiency was first described in cultured bronchial epithelial cells and bronchoalveolar lavage (20) cells stimulated with rhinovirus *in vitro* (21). Recent studies have shown normal or elevated interferon responses in mild asthma (22), in children with asthma (23) and in unchallenged severe asthma subjects (24). Type 2 inflammation is a major feature of asthma, with involvement of mast cells and eosinophils, and an expanding pathway of epithelial and lymphoid cytokines and chemokines. This has led to the development of

biologic therapies for asthma including those targeting IL-5 and IL-13 (25, 26), since these cytokines are prominent in the eosinophilic phenotype of asthma that has type-2 inflammation (27, 28).

### **Influenza vaccines: LAIV versus inactivated vaccines**

There is a major ongoing effort to improve the efficacy of influenza vaccines (2). Indeed, there is a concerted effort to develop 'universal' influenza vaccines, which would provide broad protection against many influenza strains (29). However, antigenic drift and the high number of potentially circulating strains means that current vaccines are a 'best guess' of which strains are likely to cause disease in humans, and this must be modified each year (2, 3).

Intranasal vaccination against influenza virus is a favoured site for induction of mucosal and systemic responses (3). This is because the nose is the natural site of influenza infection, involving the respiratory pseudostratified epithelium, and immune responses in the local lymphoid tissue of Waldeyer's ring: the adenoids and tonsils, with cervical lymph nodes (4). LAIV has been developed for use in infants and young children (5, 6) and is also licensed for use in adults up to 49 years of age in the USA (7). However, in adults the lower efficacy of LAIV relative to intramuscular inactivated influenza vaccine has resulted in LAIV only being licensed in the UK between 2-17 years of age (7, 30). Despite this, LAIV is frequently given to adults in the USA with an excellent safety profile (31, 32). This study will vaccinate healthy young adults (18-30 years) with LAIV.

### **Viral Load**

Following LAIV infection shedding of the vaccine virus has been detected in nasopharyngeal swabs in children (8, 9). However, shedding of the vaccine virus has not been characterised in detail, and the contribution of viral load (the extent to which the virus replicates in the airway) to vaccine efficacy is unknown. The immune response to influenza is based on both respiratory mucosal and systemic factors: in an interaction between the innate immune response of the respiratory epithelium mucosa, coupled with mucosal and systemic specific antibody (B cell) and T cell responses (10, 11). After the 2009 H1N1 influenza epidemic it was found that CD8+ T cells specific to conserved viral epitopes gave cross-protection against symptomatic influenza (12).

Quantitative PCR (qPCR) against the 4 influenza viral sub-types is analysed using a fast throughput multiplex assay to measure influenza viral load. This has been developed by Medimmune (Gaithersburg, MD) with Virosciences (Rotterdam, NL). Selection of influenza sub-type is based on WHO recommendations for influenza vaccination (2017/18) - LAIV Quadrivalent 2017/18 contained H1N1 A/Slovenia/2903/2015, H3N2 A/New Caledonia/71/2014, B/Phuket/3073/2013 (B/Yamagata-lineage) and B/Brisbane/60/2008 (B/Victoria-lineage) (33). These component vaccine strains are changed each year, in response to predictions of circulating influenza strains.



For a protective immune response to be generated to LAIV, the vaccine viruses must infect the cells of the upper airway (called ‘vaccine take’). This is believed to be lower in adults than children due to pre-existing immunity to other influenza strains, which can take the form of antibodies in both the blood and the airway mucosal fluid. However, immune correlates of vaccine take are poorly defined for LAIV.

### **Antibodies against Influenza**

A key protective mechanism of vaccination is the induction of antibodies, which participate in the prevention of future infection with pathogenic influenza. Antibody titre and function against influenza can be determined in a number of ways:

- **ELISA for antibodies**

Influenza subtype-specific IgA and IgG anti-HA antibodies can be measured by ELISA. Nasosorption using SAM strips enables higher detectable levels of total and H1-specific IgA when compared to flocced swabs or nasal wash samples (34). Following nasal LAIV this method allows the establishment of the magnitude and kinetics of nasal HA IgA antibody production.

- **Fast throughput multiplex immunoassay, based on MSD (developed by Medimmune, Gaithersburg)**

MSD immunoassays have been created to allow highly sensitive measurement of the concentration of antibody versus HA and NA recombinant antigens from the 4 vaccine virus subtypes. This is determined as the concentration of influenza viral subtype-specific IgG and IgA in blood and nasal samples. In comparison to traditional ELISA, MSD assays require less sample volume and provide faster results with higher sensitivity and specificity.

- **Haemagglutination inhibition assay (HAI)**

Protective serum humoral immunity after natural viral infection can be assessed by HAI, as routinely performed by Public Health England (Colindale, UK) (13-15), although serum levels are not used to select subjects for LAIV vaccination. Seroconversion measuring influenza HA antibodies in the HAI assay is commonly assessed after vaccination. In an integrated review of LAIV experience in over 10,000 adults and children, it was found that serum HAI geometric mean fold rises (GMFR) for HAI antibodies were higher in children and higher in baseline seronegatives (35). However, HAI responses were modest overall, and post-vaccination HAI titres were below those associated with protection for inactivated influenza vaccines.

- **Pseudotype virus neutralisation assays**

Pseudotypes and chimeric viruses utilised in assays are shown to be highly efficient for the study of cross-protective responses against multiple influenza subtypes (36). In combination

with HAI, the late nasal mucosal and serum antibody response can be investigated. Pseudotype-based antibody neutralisation assays are preferred to microneutralisation test because they are cheaper, more standardised and their greater sensitivity suits them to measurements of mucosal samples.

- **Antibody dependent cellular cytotoxicity (ADCC)/Antibody dependent effector cell activation (ADCA)**

The ability of an antibody to drive cytotoxicity of infected cells, through the activation of immune cells including Natural Killer (NK) cells and neutrophils, is a key function of antibodies against influenza (37). Techniques exist to measure this ability of antibodies from the serum (38, 39) and such techniques may be used to determine mucosal antibody function.

- **Antibody dependent phagocytosis**

Antibodies can also induce the phagocytosis of targets through antibody dependent phagocytosis (39). This property may also play a role in viral clearance and prevention of re-infection with influenza.

However, little is known about the role of local nasal mucosal immune responses to nasal vaccines and natural influenza infection, because virus load and the IgA immune response is generally measured in nasal lavage samples that are variably diluted (16-18). Hence, for LAIV nasosorption mucosal antibody measurements may be more relevant than serum HAI, and mucosal antibody measurements will be enabled by sampling of neat secretions by nasosorption.

### **Innate Immunity**

Innate immune responses occur in the first few hours after intranasal LAIV, cytokines of this immune response, particularly interferons (IFN), may cause systemic adverse events that characterise a viral illness: fever, headache and malaise (6). In older adults, proinflammatory responses may be decreased by IL-10, causing decreased immune response to vaccines and lower efficacy (19).

Study of nasal and bronchial mucosal inflammatory responses during rhinovirus-induced-asthma exacerbation has shown asthmatics presented robust anti-viral responses: including elevated nasal IFN- $\gamma$ , IFN- $\lambda$ /IL-29, CXCL11/ITAC, CXCL10/IP10 and IL-15 (40). In addition there is elevation of nasal IFN- $\gamma$ , IL-1 $\beta$ , CCL5/RANTES and IL-10 in RSV bronchiolitis of infancy (41).

### **Systems Biology**

In recent years systems biology approaches have been used to investigate seasonal influenza infection in humans: involving integrated analysis of genetics, gene expression and proteins (21). This concept has been extended to studying blood systemic vaccine responses, regarding the vaccination as an “experimental challenge model” (22). However, these

authors are clear on the future priority: “We emphasise here the paramount need for future studies deciphering the role of innate immune response to influenza virus at the site of infection” (23, 24). In our proposed study on LAIV vaccination, we propose to study nasal mucosal antibodies, the IFN response and viral load.

### **Nasal Lavage**

Nasal lavage has been extensively used in the study of patients with allergic rhinitis (42), and also to measure nasal mucosal responses to intranasal LAIV (16, 17). However, nasal lavage with 3.0 to 5.0ml saline has the problems of dilution of secretions, variable recovery, and not being able to repeat for 24h for repeatable results (43). Nasal lavage shortly following vaccination also risks washing the vaccine virus out of the nose, preventing infection. This technique is therefore poorly suited to investigating the innate immune response to influenza. Levels of mediators in nasal lavage are uniformly lower than after nasosorption (34, 44, 45); we are therefore interested to study levels of antibodies and inflammatory mediators in alternative respiratory samples.

### **Nasosorption**

The nose is much more accessible than the airways to obtain respiratory samples (46), and nasal and bronchial epithelial mucosal lining fluid (MLF) is a potentially important sample to study inflammatory mediators in a variety of respiratory diseases: ranging from a variety of infections, to chronic conditions including asthma, chronic obstructive pulmonary disease (COPD), interstitial lung diseases (47) and lung cancer.

Strips of synthetic absorptive matrices (48) or synthetic sponge can be used to sample MLF, before eluting the fluid and measuring levels of mediators that cause and are a feature of inflammation. Both natural and synthetic sponges have been used to absorb MLF from mucosal surfaces. Ophthalmic Weck-Cel sponges composed of natural cellulose have been used to sample saliva, cervical and vaginal secretions (47, 49). Different absorptive materials have been compared for sampling oral fluid prior to measuring antibodies (50), while polyurethane minisponges have been used to collect human tears (51).

Filter paper or natural cellulose from the cotton plant has been widely used to absorb nasal secretions (52-56). Nasal lavage has been used to detect IL-5 and IL-13 in nasal secretions after nasal allergen challenge (17, 57, 58). However, nasosorption with filter paper detected IL-5 and IL-13 at higher levels (59). Following this it was found that different batches of filter paper vary in their degree of protein binding, some failing to release cytokines (internal data), and thus we decided to identify a suitable synthetic absorptive matrix (SAM).

SAMs are now generally used to obtain nasal MLF by nasosorption (44, 48). These absorbent materials are comfortable to use and can obtain MLF even from inflamed noses at frequent intervals over extended periods of time. The eluate from nasosorption contains cytokines and chemokines at high detectable levels by immunoassay. SAMs have been utilised for

nasosorption in children with allergic rhinitis (60), infants with a family history of atopy (61), and in atopic adults after nasal allergen challenge (17) when employing therapeutic anti-IL-13 monoclonal antibodies (48). A validation study has used a variety of SAMs after NAC to show tryptase, eosinophil cationic protein (ECP) and IL-5 responses (62). In contrast, a study with nasal LPS challenge showed IL-1 $\beta$  and IL-6 responses (63). In relation to experimental human rhinovirus (HRV) challenge in asthma, nasosorption was performed to measure IL-15 (64), IL-25 (65), IL-33 (66) and IL-18 (67).

Nasosorption devices are now manufactured by Hunt Developments, a specialist medical device company from Midhurst in West Sussex. They have clean rooms that ensure nasosorption medical devices can be prepared free from lipopolysaccharide (LPS), dust and microbial contamination. Nasosorption devices are CE (Conformité Européenne)-marked as Class I medical devices. Good Manufacturing Practice (GMP) is followed in a specialised medical device facility; with medical grade materials throughout, ensuring devices are sterile, LPS and dust free.

This study proposes the use of precision mucosal sampling through nasosorption, in which a synthetic absorptive matrix (48) is used to absorb nasal mucosal lining fluid (MLF). In previous studies in asthma and hay fever we found that nasosorption sampling permits higher levels of mediators to be detected than can be found in nasal lavage. Nasosorption can also be repeated with high frequency, up to every few minutes (62), permitting detailed investigation of the kinetics of the immune response. Studies have indicated that nasosorption can be used to perform viral RNA analysis by reverse transcriptase-polymerase chain reaction (RT-PCR) to determine viral load (41).

## **Project Impact**

- **Mucosal sampling and assays**

There is a paramount need to study local nasal mucosal immune responses to nasal LAIV, and this will depend on the development of nasal sampling methods and sensitive assays for assessing the virus and the immune response.

- **Biomarkers and correlates of influenza LAIV Responses and Protection**

It is thought that the immune response to LAIV is mediated by a combination of mucosal and systemic factors, involving innate and specific mechanisms that have different kinetics and various cell types. Through understanding the molecular and cellular basis of the nasal mucosal response to LAIV, we hope to be able to identify key molecular signatures and biomarkers that correlate with LAIV responses, and to assess protective pathways that could be stimulated by novel vaccines.

- **The nasal vaccine challenge model**

The nasal vaccine challenge model could be developed to test new vaccines, and proceed to rational development of improved vaccines for influenza and other diseases. Advanced mucosal sampling, assays and biomarkers could be used to accelerate clinical assessment of vaccine development.

- **Clinical applications in vaccinology**

Establishing correlates of protection and vaccine take may identify subjects who have responded poorly to vaccines, and allow assessment of vaccine efficacy in large populations including patients that are immunocompromised and vulnerable to influenza. Establishing correlates of protection may enable vaccines to be targeted to patients who require protection. Use of standardised agents (such as vaccine-grade attenuated vaccine viruses) and standardised techniques (such as nasosorption) the feasibility of large, multi-centre studies is greatly enhanced. These features could permit studies of vaccine efficacy across countries, ethnicities and other population demographics.

## Study Objectives

In our proposed study of intranasal LAIV we are especially interested in a number of aspects in relation to **vaccine take and correlates of efficacy**:

1. Characterise nasal MLF IgA/G responses to specific viral sub-types in terms of the kinetics and magnitude of the immune response to LAIV specific influenza sub-type antibodies: measured using ELISA and by MSD multiplex immunoassays
2. Quantify serum IgG antibody to 4 influenza sub-types: measured using ELISA and by MSD multiplex immunoassay
3. Determine serum and nasal (if feasible) HAI to all 4 influenza vaccine viruses
4. Determine serum and nasal (if feasible) antibody neutralisation of HA coated pseudotype viruses (representing the 4 influenza vaccine viruses)
5. Quantify antibody dependent phagocytosis (ADP) of HA bearing particles using serum and MLF antibodies
6. Develop antibody dependent cellular cytotoxicity (ADCC) and antibody dependent cellular activation (ADCA) assays using serum and MLF antibodies
7. Quantify viral load (all 4 strains in the vaccine, by qPCR)
8. Innate IFNs and IFN-related proteins
9. Determine the relationship between local and systemic immune responses
10. Determine the relationship between innate and adaptive immune responses
11. Explore predictors of the response (responders): Mediators & cellular parameters that predict infection and/or antibody induction

## Hypotheses

1. Influenza sub-type MLF IgA antibodies from the nose can be quantified by ELISA and MSD multiplex immunoassays and functional characteristics determined (e.g. neutralisation, HAI, ADP, ADCC/ADCA)
2. Nasosorption can be used to follow the kinetics of LAIV influenza sub-type load (by qPCR)
3. Baseline and early innate-immune induced cytokines (interferons) and chemokines (especially relating to IFN responses) will affect the specific immune responses to LAIV
4. Pre-existing antibody immunity (serum or mucosal IgG or IgA) against the vaccine influenza sub-types prevent infection and/or decrease viral shedding load/duration

## Aims:

1. Development of a clinical testing model to assess **LAIV correlates of efficacy**:
  - a. Validation of novel sampling methods for the quantification of viral load and antibody measurements
  - b. Determination of biomarkers of the innate and adaptive immune response to LAIV

- c. Determine the influence of pre-existing immunity and the early innate response on viral take and the ensuing specific immune response
- 2. To provide a proof-of-concept human clinical testing model for **novel vaccine approaches**:
  - a. To improve tools and provide biomarkers for the rational design and improvement of respiratory mucosal viral vaccines
  - b. For testing different influenza vaccine strains (selected for the upcoming season) and attenuated vaccines with different formulations (e.g. nanomaterials) and adjuvants.

## Study Design

### Participants and Recruitment

Participants will be recruited from Imperial College London through local advertisement for participants aged 18-30. ICRRU also holds a database of participants who have previously given their contact information and consented to be contacted about future ethically approved studies. This database will also be used to contact potential participants with advertisement materials. For the pilot study and the main vaccination study the following groups will be recruited:

- Non-asthmatic participants with allergy (atopics); n=15 (pilot study only)
- Asthmatics; n=15 (pilot study only)
- Healthy volunteers without allergies or asthma; n=55 (including n=15 for pilot study\*)

\*Healthy volunteers who participate in the pilot study **will** be eligible to participate in the main vaccination study.

## Study Outcome Measures

### Primary Outcome Measure

Induction of nasal mucosal antibodies to the vaccine influenza strains, defined by a four-fold or greater rise in antibody titre.

### Secondary Outcome Measures

1. Serum levels of antibodies against the 4 constituent viral subtypes in LAIV: measured by ELISA and multiplex immunoassay (Mesoscale Diagnostics)
2. Haemagglutination inhibition (HAI) titres against the 4 viral subtypes from day 0 to day 28 measured in serum and the nose.
3. Neutralising antibodies (measured by pseudotype assays) measured in the serum and nose against the 4 vaccine virus subtypes from day 0 to day 28
4. Elevation in antibody effector function (serum and nasal antibodies): Including ADCC/ADCA and ADP
5. Mucosal innate immune response: nasal MLF cytokines and chemokines generated within the first 8-hours post-vaccination. Including: IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IFN- $\lambda$  (IL-29), IP-10 and ITAC
6. Mucosal LAIV viral load in the first week after vaccination



7. Association of transcriptional signatures (nasal and blood) with induction of antibodies.

## **Participant Entry**

### **Pre-Enrolment Evaluations**

At each participants screening visit their medical history, details of any drugs/medications used and a physical assessment will be performed. These investigations will seek to rule out the presence or history of any respiratory or other health conditions which meet the exclusion criteria. An ECG will additionally be performed to exclude the possibility of any underlying cardiovascular condition. A urine pregnancy test will be performed on all female participants, as pregnancy may influence the study results.

### **Inclusion Criteria**

- Capacity to provide informed consent.
- Aged 18-30 years - selected to increase likelihood of 'vaccine take' and minimise risk.
- Fluent English speaker, to ensure comprehensive understanding of study aims, methodologies and outputs.

### **Exclusion Criteria**

- Current involvement in another study unless observational or in follow-up phase (non-interventional).
- Received any influenza vaccine over the last 2 years.
- Egg allergy (as per influenza vaccines patient leaflet).
- Previous significant adverse reaction to any previous vaccination/immunisation.
- Current regular (daily) smoker.
- Pregnant - as this may influence the study results.
- Taking medication that may affect the immune system in any way e.g. steroids, steroid nasal spray.
- Regularly taking acetylsalicylic acid (aspirin) - as per LAIV guidance to reduce the risk of Reye's syndrome.
- Unable to give fully informed consent.
- Current acute severe febrile illness - to avoid vaccination and inoculation in participants that may have current infection.
- Taking long term antibiotics e.g. following splenectomy or sickle cell disease.
- Clinically diagnosed with flu in the last 2 years.

- A long-term health problem with heart disease, lung disease (including asthma), kidney disease, neurologic disease, liver disease, metabolic disease (e.g., diabetes), or anaemia or another blood disorder (LAIV contraindications list).
- Use of drugs for the treatment of rheumatoid arthritis, Crohn's disease, or psoriasis or anticancer drugs; or radiation treatments (LAIV contraindications list).
- History of Guillain-Barré syndrome
- Live with or expect to have close contact with a person whose immune system is severely compromised and who must be in protective isolation (e.g., an isolation room of a bone marrow transplant unit)
- Received any other vaccinations in the past 4 weeks.

### **Withdrawal Criteria**

In the event of a serious adverse event (SAE), vaccination visits will all be postponed. The cause of the SAE will then be investigated. If the SAE is considered to result from the study protocol then the study will be terminated. Participants will be vaccinated in groups of maximum 3 on any single day. At most 6 will be vaccinated in any single week.

Participants will be free to withdraw from the study at any point. Any samples collected up to the point of their withdrawal will be retained for analysis in anonymised form.

### **Randomisation and Enrolment Procedures**

As this is an investigational study assessing the mechanism underlying immune protection conferred by vaccination, and not a trial of efficacy, no randomisation of participants will be performed. Additionally, the study is not placebo controlled or blinded and will be conducted as an 'Open Label' study. All participants enrolled to the main vaccination study will therefore receive LAIV.

### **Treatments**

All participants in the main vaccination study (not the pilot study) will receive the same LAIV. The formulation of this vaccine is altered each year to reflect the circulating strains of influenza in that year. This information is not released until May or June each year, so the exact formulation of LAIV for 2018/19 is not presently known. However, the vaccine typically contains  $1 \times 10^7$  fluorescent focus units of each of the four vaccine virus strains. The vaccine will be administered intra-nasally in 100µl volumes to each nostril. Participants breathe normally during the vaccine administration, which takes less than one minute.

LAIV for this study will be sourced from OTC direct clinical trials services ltd who are the registered distributor of LAIV for clinical trials in the UK. This will be received in its normal packaging for use in the UK market, which will not be altered prior to vaccination. The investigators will pay OTC for LAIV. Considerable safety data is available from young adults immunised with LAIV in countries, such as the USA, where LAIV is licensed for this population. As such, no dose modification will be conducted.

### **Premedication**

No drugs will be prescribed before or during the trial.

### **Interaction with other drugs**

Fluenz Tetra/FluMist should not be given in conjunction with salicylate (aspirin) therapy due to the reported associations between use of salicylates and wild type influenza with Reye's syndrome.

Antivirals such as Oseltamivir should not be used during the trial due to suppression of vaccine replication, though no adverse safety events have been reported.

Efficacy of other live attenuated vaccines (e.g. Yellow Fever Vaccine) could be influenced by Fluenz Tetra/FluMist, though no clinically meaningful changes have been observed. As such we ask participants to discuss any planned live attenuated vaccinations with us during their involvement in the study.

### **Dispensing and Accountability**

LAIV will be kept in a monitored fridge on ICRRU throughout the study duration and will be accounted for and administered by the study physician.

## Adverse events

### Definitions

**Adverse Event (AE):** any untoward medical occurrence in a patient or clinical study subject.

**Serious Adverse Event (SAE):** any untoward and unexpected medical occurrence or effect that:

- **Results in death**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation**
- **Results in persistent or significant disability or incapacity**
- **Is a congenital anomaly or birth defect**

Medical judgement should be exercised in deciding whether an AE is serious in other situations. Important AEs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

### Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the Chief Investigator in the first instance.

#### Non serious AEs

All such events, whether expected or not, should be recorded.

#### Serious AEs

An SAE form should be completed and faxed to the Chief Investigator within 24 hours. However hospitalisations for elective treatment of a pre-existing condition do not need reporting as SAEs.

All SAEs should be reported to the London Camberwell St Giles REC where in the opinion of the Chief Investigator, the event was:

- 'related', i.e. resulted from the administration of any of the research procedures; and
- 'unexpected', i.e. an event that is not listed in the protocol as an expected occurrence

Reports of related and unexpected SAEs should be submitted within 15 days of the Chief Investigator becoming aware of the event, using the NRES SAE form for non-IMP studies. The Chief Investigator must also notify the Sponsor of all SAEs.

Local investigators should report any SAEs as required by their Local Research Ethics Committee, Sponsor and/or Research & Development Office.

**Contact details for reporting SAEs and SUSARs:**  
**r.nicholson@imperial.ac.uk**  
**CI email: p.openshaw@imperial.ac.uk**  
**Fax: 02033125751, attention Peter Openshaw**  
**Please send SAE forms to: Peter Openshaw by any of the**  
**above methods**  
**Tel: 0207 594 3854 (Mon to Fri 09.00 – 17.00)**

## **Assessments and Follow-ups**

The study comprises two phases, detailed below:

- 1) A single visit pilot study, which does not involve LAIV administration and no follow up.
- 2) The main 6 visit study, with administration of LAIV. If these participants are lost to follow up their loss will not be flagged. The existing excellent safety profile of LAIV makes any such loss to follow up unlikely to result from the vaccine.

## **Trial Closure**

The trial will be closed at the end of the study period (29/05/2020). All study documents will be retained for a period of no-less than 10 years after trial closure.

## **Statistics and Data Analysis**

As a study of the mechanism of action of LAIV, rather than a trial of efficacy, the endpoints and planned statistical assessments are exploratory. The sample size calculation is based on an estimated sero-conversion rate of 50%, with a documentable infection with vaccine virus(es) expected in 30% of participants (though this data is incomplete).

## **Pilot Study: Nasal Sampling Methods and Measurement of Nasal Mucosal Mediators and Antibodies**

This Preliminary Pilot Study is carried out with the aim of selecting and validating the optimal methods of sampling and analysis for the main study. The pilot does not involve LAIV vaccination or physical examination.

*Subjects:* We shall undertake a Preliminary Pilot Study in volunteers (n=45), including atopics (n=15), asthmatics (n=15) and healthy volunteers (n=15).

### *Patient Information and Consent*

We shall have a separate Patient Information Sheet and Consent Form for this Preliminary Pilot Study. Live cells will be retained, along with samples for transcriptional studies (RNA), but not DNA.

### *Samples:*

#### 1. Blood sampling

We shall take a single blood sample of up to 50ml blood: for optimisation of cellular immune and antibody assays.

#### 2. Gum crevicular fluid

This will be collected using a specially designed Oracol swab.

#### 3. Nasal Sampling Methods:

- Nasosorption: We shall compare different methods of nasosorption of nasal MLF involving different medical grade materials, including poly-urethane and Leukosorb.

A sampling schedule will be performed as follows, in order to establish repeatability and reproducibility of measurements:

- Left nostril: 0min, 30min
- Right nostril: 0min, 30min

### Sample elution methods:

We shall compare methods for elution of nasosorption samples with a variety of elution buffers and manual or centrifugation methods.

- Nasal wash
- Nasal curettage: We shall compare different methods of nasal microcurettage: Rhinoprobe versus CellSkim. We shall take up to 4 microcurettage specimens per volunteer.

### *Assays:*

We shall measure markers of inflammation (e.g. cytokines and chemokines), mucosal antibodies, microRNA, mRNA, the microbiome and viral RNA. In particular we shall optimise techniques for the measurement of influenza IgG and IgA antibodies (nasal and serum) and their function.

## **Main Study: Nasal Mucosal and Systemic Immune Responses to Intranasal LAIV in Healthy Adults of 18-30 years**

*Subjects:* The main study will recruit healthy volunteers (n=40) through local advertisement within Imperial College London.

### *Patient Information and Consent*

We shall have a separate Patient Information Sheet and Consent Form for this Main Study. Live cells will be retained, along with samples for transcriptional studies (RNA), but not DNA.

### **Primary End-Point**

Induction of nasal mucosal antibodies to the 4 constituent viral subtypes in LAIV: quantified by ELISA and multiplex immunoassay (Mesoscale Diagnostics) and expressed as seroconversion, geometric mean titre (GMT) and geometric mean fold rises (GMFR).

### **Secondary End-Points**

1. Serum levels of antibodies against the 4 constituent viral subtypes in LAIV: measured by ELISA and multiplex immunoassay (Mesoscale Diagnostics)
2. Haemagglutination inhibition (HAI) titres against the 4 viral subtypes from day 0 to day 28 measured in serum and the nose
3. Neutralising antibodies (measured by pseudotype assays) measured in the serum and nose against the 4 viral subtypes from day 0 to day 28
4. Antibody effector function (serum and nasal antibodies): Including ADCC/ADCA and ADP
5. Mucosal innate immune response: nasal MLF cytokines and chemokines generated within the first 8-hours post-vaccination. Including: IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IFN- $\lambda$  (IL-29), IP-10 and ITAC
6. Mucosal LAIV viral load in the first week after vaccination
7. Association of transcriptional signatures (nasal and blood) with induction of antibodies.

### **Schedules for Sampling**

For reference, also see **appendix 1** of this document.

### **Sampling at screening visit**

The screening visit will firstly assess candidates for eligibility, based on the study exclusion criteria and contraindications for LAIV vaccination, including completion of checklist for LAIV contraindications. This screening visit should take place 1-6 weeks prior to the vaccination visit. Once this screening has been completed, the following samples will be collected from volunteers:

- Nasosorption sampling

- Nasal wash
- Nasal curettage
- Blood samples (less than 50ml)
- Gum swab using Oracol swabs

If no contraindications for LAIV administration are found then participants will be invited to proceed to the main vaccination study.

### **Sampling schedule in relation to LAIV administration on Day 0 (0d)**

For reference to the study schedule on the vaccination day (0d), see **appendix 2**:

- Nasosorption sampling on vaccination day (0d): pre-vaccine, 3h, 4h, 5h, 6h, 7h, 8h
- Nasosorption sampling on days after 0d: 1d, 3d, 7d and 28d
- Nasal curettage: 3d
- Nasal wash: 28d
- Blood samples will be collected at 1d, 3d, 7d and 28d

### **Clinical Assessments at study visits**

Physical assessment of symptoms and Nasal Inspiratory flow will be determined once on each of the following visits: Screening, 1d, 3d, 7d and 28d.

On the vaccination visit 2 (0d), physical assessment of symptoms and nasal inspiratory flow will be determined immediately following nasosorption sampling at times: pre-vaccine, 4h, 8h.

Physical examination will involve a general examination of participant's nose, heart and lungs. This will include taking a history, measuring blood pressure, heart rate, breathing rate and temperature.



## Nasal and Serum sample assays

Samples may be shipped directly to international collaborating study partners or contract research organisations for analysis, the patient information sheets and consent forms for this study highlight this point.

Samples arising from this study will be used for laboratory experiments, including:

- **Viral Sub-Type qPCR: 4 Influenza strains in Flumist (Nasal only)**  
Fast throughput qPCR assay developed through Medimmune (Gaithersburg, USA) and Virosciences (Rotterdam, NL).  
qPCR primers available for the 4 sub-types designated by the WHO for 2018/19
- **Cytokines/chemokines:**  
IFN- $\alpha$ , IFN- $\beta$ , IFN- $\gamma$ , IFN- $\lambda$  (IL-29), IP10, ITAC  
IL-1 $\beta$ , IL-6  
IL-5, IL-13, IL-33

## Antibody Assays: Serum and Nasal samples

### 1. Viral sub-type specific IgG and IgA versus HA

High sensitivity immunoassay for the quantification of antibodies against each vaccine strain; developed by Medimmune. To be performed on serum and nasal respiratory samples.

### 2. HAI serum IgG haemagglutination inhibition antibodies

*In collaboration with Katja Hoschler, Public Health England*

HAI functional assays for the 4 individual vaccine virus subtypes.

- Seronegative titre <8, seropositive titre >8
- Serum response defined as >4 fold rise
- Geometric mean fold rises (GMFRs)

Performance of respiratory samples (nasosorption or nasal wash) unknown: detergent in the samples could interfere with the assay, development is therefore needed.

### 3. Pseudotype neutralisation antibodies

*In collaboration with Nigel Temperton, University of Kent*

- Replication-defective HIV core, integrated with HA subtype on virion surface, expresses luciferase upon infection: high sensitivity and large dynamic range compared to classical neutralisation assays.
- Available viral subtypes are close to the Flumist: H1N1 (California) and H3N2. New pseudotypes can be rapidly generated.
- Requirement for whole HA RNA sequences, which are available online (33): ordered from Genscript HK (or Invitrogen) for cloning.
- Determines neutralising antibody concentrations.

Performance of respiratory samples (nasosorption or nasal wash) unknown: detergent in the samples could interfere with the assay, development is therefore needed.

#### **4. Antibody dependent cellular cytotoxicity (ADCC)/Antibody dependent effector cell activation (ADCA)**

Antibodies can drive the killing of infected cells, a key mediator of protection (37). Techniques exist to measure this ability of antibodies from the serum (38, 39) and such techniques could be used to determine mucosal antibody function.

- Antibody dependent cytotoxicity assays require target cells to either be infected by the pathogen of interest, or to express a target antigen (e.g. a HA variant). The assay then determines lysis of these target cells by immune effector cells, in an antibody dependent manner (38, 39).
- Alternatively antibody dependent effector cell activation (ADCA) assays look at activation of effector cells, but do not measure lysis of a target cell. Instead they measure expression of a surface activation marker, or secretion of an effector protein. These assays therefore do not require target cells and can be performed with recombinant proteins (38, 39).

Performance of respiratory samples (nasosorption or nasal wash) unknown: detergent in the samples could interfere with the assay, development is therefore needed.

#### **5. Antibody dependent phagocytosis**

Antibodies can also induce the phagocytosis of targets through antibody dependent phagocytosis (39). This property may play a role in viral clearance and prevention of re-infection with influenza (16-18), but has not been studied in respiratory mucosal antibodies.

Performance of respiratory samples (nasosorption or nasal wash) unknown: detergent in the samples could interfere with the assay, development is therefore needed.

## **Data Analysis: Bioinformatics and Systems Biology**

### **Systemic and mucosal vaccine systems biology**

Systems biology approaches have been introduced to looking at seasonal influenza infection in humans (21), and also for blood systemic vaccine responses, regarding the vaccination as an “experimental challenge model” (22-24). There is now the opportunity to perform systems biology on both the blood (systemic) and nasal mucosal (local) immune response to LAIV. This is because in this proposed study on LAIV vaccination, we propose to primarily study nasal mucosal and serum antibodies, the innate immune response and viral load. However, there will also be assessment of the transcriptional gene expression response (systemic (blood) and mucosal) to LAIV. Integrating these transcriptomics data with other data arising from this study (antibody generation, function, viral load and the innate immune response) a complete view of the immune response to influenza can be generated. This approach will further the ability to distinguish responses to infection and correlates of vaccine efficacy.

### **Responders and non-responders**

Our primary postulated correlate of influenza protection is the nasal MLF level of influenza-specific IgG and IgA. Following LAIV we would like to classify our subjects in terms of nasal mucosal IgG and IgA geometric mean fold rises (GMFR): or nasal mucosal “responders” and “non-responders”. However, since our study is exploratory we will have to assess the kinetics and magnitude of the nasal mucosal immune response before we can provide this definition. Other important post-LAIV vaccine responses will be the magnitude of the initial mucosal innate immune response and the nasal viral load.

### **Predictors of the Response**

It will be necessary to construct predictive models to then postulate those indices pre- and post-vaccination which correlate with protection. These generated predictors of response would need to be validated in a subsequent cohort or in the context of influenza live viral challenge or natural infection.

#### **Before LAIV:**

We postulate that nasal antibody levels inversely correlate with subsequent local and systemic IgG and IgA responses.

There may be molecular signatures in nasal MLF, nasal curettage and blood that will predict the nasal and systemic antibody responses to LAIV.

#### **Following LAIV:**

We postulate that the size of the initial nasal innate immune response and viral load may predict the magnitude or functionality of the subsequent nasal antibody response.

There may be molecular signatures in nasal MLF, nasal curettage and blood following LAIV that will predict (1) the nasal and systemic HAI IgG/IgA responses to LAIV (2) protection against influenza.

## **Risks and Benefits to Study Participants**

In the USA this vaccine is routinely administered to adults with excellent safety. As such we do not anticipate any adverse reactions to vaccination beyond mild symptoms to influenza like illness, such as malaise, fever and respiratory tract symptoms. Even such symptoms are rare. This study has received written confirmation from the Medicines and Healthcare products Regulatory Agency (MHRA) that the study does not require registration as a clinical trial of an investigational medicinal product, despite there not being a marketing authorisation for this product in the UK.

The participants will benefit through the studies administration of an influenza vaccine, which may protect them from infection. However, we will request that the participants do not receive any other influenza vaccines until after the final study visit. As LAIV has a lower protective efficacy than intramuscular inactivated influenza in this group of young adults, the participants may be at higher risk of influenza infection than if they had elected to receive an inactivated influenza vaccine. This risk is minimal, as vaccination is not routinely recommended to this population. Additionally, at the end of the study we will ensure that participants are informed about available influenza vaccines, should they decide that they would like an additional vaccination.

Participants will receive remuneration for their time and travel costs associated with the study. For the pilot study (single one hour visit) participants will receive £25. For the main LAIV study (6 visits, totalling 13 hours) participants will receive £325.

## **Monitoring**

### **Risk Assessment**

Given the excellent safety profile of LAIV in the target population in other countries, including an FDA license for the USA, and the marketing authorisation in the UK for infants, the study is considered low risk. As such a minimum level of monitoring will be required by the study and will be performed between the study team and the Joint Research Compliance Office of Imperial College London.

### **Monitoring at ICRRU**

Due to the low risk nature of the trial monitoring visits will not be routinely scheduled, but will be available for monitoring as required.

## **Regulatory Issues**

### **Clinical Trials Authorisation**

This study has received written confirmation from the Medicines and Healthcare products Regulatory Agency (MHRA) that this study does not require registration as a clinical trial of an investigational medicinal product. As such, review for approval by the MHRA is not required.

### **Ethics Approval**

The Study Coordination Centre has obtained approval from the London Camberwell St Giles Research Ethics Committee (REC) and Health Regulator Authority (HRA). The study will also receive confirmation of capacity and capability from the Imperial College NHS Healthcare Trust before accepting participants into the study or any research activity is carried out. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

### **Consent**

Consent to enter the study will be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent will be obtained. The right of the participant to refuse to participate without giving reasons will be respected. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing future treatment.

## **Confidentiality**

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

## **Indemnity**

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

## **Sponsor**

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

## **Funding**

The National Institute for Health Research (NIHR) Imperial Biomedical Research Cluster (BRC) are funding this study.

## **Audits and Inspections**

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor and other regulatory bodies to ensure adherence to GCP.

## **Publication Policy**

The results of the trial will be published in scientific journals, presented at conferences as oral and poster presentations and distributed to collaborators. No patient identifiable information will be included in any publication or disseminated material.

## APPENDIX 1: Main Study Sampling Schedule

Study procedures	Visit 1 Screening	Visit 2* Nasal LAIV	Visit 3 Day 1 (24h)	Visit 4 Day 3 (72h)	Visit 5 Day 7	Visit 6 Day 28 (26-30)
	1 hour visit	Full day visit	1 hour visit	1 hour visit	1 hour visit	1 hour visit
Assessment of Contraindications for LAIV	X					
Vaccination with Nasal LAIV		X				
Medical History	X					
Physical Examination	X	X	X	X	X	X
ECG	X					
Urine pregnancy test	X					
Blood Test	X		X	X	X	X
Skin Prick Test	X					
Nasal Inspiratory Flow	X	X	X	X	X	X
Nasosorption	X	X (x7*)	X	X	X	X
Nasal wash	X					X
Nasal scrape (nasal curettage)	X			X		
Gum swab	X					X

\* - See Appendix 2 for further detail on the schedule for study Visit 2

## APPENDIX 2: Visit 2 (Vaccination day) sampling schedule

Study procedures	Time from arrival at ICRRU						
	0h	3h	4h	5h	6h	7h	8h
Vaccination with Nasal LAIV	X						
Physical Examination	X		X				X
Nasal Inspiratory Flow	X		X				X
Nasosorption	X	X	X	X	X	X	X



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